CHAPTER 2

The Biology of *Giardia* Parasites

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2.1 PREFACE

Giardia intestinalis (syn. *G. lamblia* or *G. duodenalis*) is one of the ten major enteric parasites affecting humans worldwide. It is also considered the most common intestinal pathogenic protozoa of humans and is recognized as a recurrent parasite of other nonhuman species, including cattle, beavers, and domestic dogs. Even though *Giardia* was first observed by Van Leeuwenhoek in 1681, in the past it was debated whether *Giardia* was a pathogen. However, now it is accepted that *Giardia* can cause intestinal disease in humans and in a wide range of domestic and wild mammals. The clinical presentations of giardiasis range from an asymptomatic cyst excreting state to diarrhea, which can be acute, chronic, or intermittent (Karanis *et al.*, 1996; Kirkpatrick and Benson, 1987; Monzingo and Hibler, 1987; Nizeyi *et al.*, 1999; Olson *et al.*, 1995; Pacha *et al.*, 1985; Patton and Rabinowitz, 1994; Rickard *et al.*, 1999; Sulaiman *et al.*, 2003; Thompson *et al.*, 2000; Wallis *et al.*, 1984; Xiao, 1994). Recently, *Giardia* infections have been associated with growth faltering due to nutrient malabsorption (Berkman *et al.*, 2002).

Giardia intestinalis is a parasite of public health importance as it can be transmitted through several routes, including water (drinking as well as recreational) and fresh food products (Nichols, 2000). Since foodborne outbreaks occur more frequently on a smaller scale than waterborne outbreaks, they are identified less frequently. Nonetheless, there are well-documented outbreaks implicating *G. intestinalis* as the causative agent (Anonymous, 1989; Mintz *et al.*, 1993).

In developed countries, *Giardia* is currently referred as a reemerging infectious agent because of its increasing role in outbreaks of diarrhea in day-care centers, and water and foodborne outbreaks affecting the general population. However, in developing countries located in Asia, Africa, and Latin America, approximately 200 million people per year experience symptomatic giardiasis (Thompson *et al.*, 2000).

There are multiple recognized species of the genus *Giardia*, although only *G. intestinalis* is found to be pathogenic to humans. Recent genetic studies have identified distinct groups within this species (Andrews *et al.*, 1989; Meloni *et al.*, 1995), and several researchers now regard *G. intestinalis* as a species-complex (Andrews *et al.*, 1989; Ey *et al.*, 1997; Monis *et al.*, 1998) (Table 2.1). Furthermore, the zoonotic potential of some animal isolates of *G. intestinalis* has recently been suggested (Sulaiman *et al.*, 2003). These findings plus their impact on the public health emphasize the importance to understand the biology, epidemiology, transmission, control, and treatment of *G. intestinalis* parasites.

Table 2.1. *Giardia intestinalis* assemblages and host range of isolates.

Assemblage (Genotype)	*Host range*	*Reference*
A	Human, cat, dog, calf, horse, pig, deer, lemur, beaver, slow loris, guinea pig	Homan *et al.*, 1992; Maryhofer *et al.*, 1995; Meloni *et al.*, 1995 ;Monis *et al.*, 1996; Ey *et al.*, 1997; Karanis and Eye 1998; Monis *et al.*, 1999; Trout *et al.*, 2003; Sulaiman *et al.*, 2003
B	Human, dog, monkey, beaver, muskrat, chinchilla, guinea pig, rabbit	Homan *et al.*, 1992; Maryhofer *et al.*, 1995; Meloni *et al.*, 1995; Ey *et al.*, 1997; Monis *et al.*, 1999; Sulaiman *et al.*, 2003
C, D	Dog	Maryhofer *et al.*, 1995; Hopkins *et al.*,1997; Monis *et al.*, 1998; Monis *et al.*, 1999; Sulaiman *et al.*, 2003
E	Cow, goat, sheep, pig	Ey *et al.*, 1997; Sulaiman *et al.*, 2003
F	Cat	Maryhofer *et al.*, 1995; Meloni *et al.*, 1995; Hopkins *et al.*, 1999; Monis *et al.*, 1999
G	Rat	Monis *et al.*, 1999; Sulaiman *et al.*, 2003

2.2 BIOLOGY

Giardia species are flagellated unicellular enteric protozoan parasites, inhabiting the intestinal tracts of almost every group of vertebrates, causing giardiasis. Some of the infections can be asymptomatic, although diarrhea and other discomforts are commonly observed in a large number of infected animals, children, and adults. The incubation or pre-patent period is longer than bacterial or viral infections, usually lasting from 12 to 19 days (Jokipii *et al.*, 1985).

The vegetative trophozoites and the environmentally resistant cysts are the two major stages in the life cycle of *Giardia*. Infection occurs when viable cysts are ingested by a susceptible host either through contaminated water, food, or contact with contaminated materials. Within a few hours, excystation or cyst opening occurs in the proximal part of the intestine where two newly formed trophozoites are released and proceed to infect intestinal cells. The parasite adheres and multiplies on the luminal lining of the small intestine, leading to diarrhea which may interfere with nutrient absorption. After multiplication, the trophozoites pass to the terminal region of the intestine and form new cysts that are excreted in stools. The cysts are released in variable quantities, and can survive well in the environment, allowing the parasite to reach other susceptible hosts and cause new infections.

Giardia is regarded as one of the most primitive eukaryotes in existence, and is considered to be the missing evolutionary link between eukaryotes and prokaryotes by a number of evolutionary biologists (Nasmuth, 1966; Sogin, 1991). As a typical eukaryotic organism, *Giardia* parasites contain distinct nuclei with a nuclear membrane and cytoskeleton. However, some other organelles commonly present in eukaryotes, such as the nucleoli, peroxisomes, and mitochondria are absent (Adam, 2001). Additionally, *Giardia* does not reproduce sexually. These characteristics, plus

the lack of fossil records and similarities in microscopic characteristics (including morphology and ultrastructural features), make it difficult to define a species structure of *Giardia*, which can truly reflect its biologic characteristics and evolutionary relationships (Barta, 1997; Monis, 1999).

The taxonomy of *Giardia* at the species level was confused and complicated through the first half of the twentieth century. Historically, 41 species of *Giardia* with uncertain validity can be found in the literature. These reports istakenly named *Giardia* species based on the host of origin (Campbell *et al.*, 1990; van Keulen *et al.*, 1993).

At present, only six species of the genus *Giardia* are considered valid. Filice (1952) classified *Giardia* using morphological characteristics and recognized three distinct species: *G. duodenlalis* infecting a wide range of mammals, including humans, livestock, and companion animals; *G. agilis* in amphibians; and *G. muris* in rodents. In 1987, the species *G. ardeae* and *G. psittaci* were described in birds (Erlandsen and Bemrick, 1987; Erlandsen *et al.*, 1990), and shortly thereafter *G. microti* was described in muskrats and voles. This last species of *Giardia* was identified on the basis of cyst morphology and rRNA nucleotide sequence analysis (Feely, 1988; van Keulen *et al.*, 1998). Thus, *G. microti* was the first species of *Giardia* to be determined using both morphological and molecular features. This species was further validated by the characterization of nucleotide sequences of the triose phosphate isomerase (TPI) gene isolated from muskrats (Sulaiman *et al.*, 2003).

It has been recently postulated that the taxonomy of *Giardia* at the species level is still unresolved, as there are biological and molecular differences between isolates within a species; although their cysts or trophozoites morphologically identical. This is further supported by the observations of significant diversity among *G. intestinalis* isolates in host-infectivity assays (Visvesvara *et al.*, 1988), metabolism (Hall *et al.*, 1992), and *in vitro* and *in vivo* growth requirement (Andrews *et al.*, 1992; Binz *et al.*, 1992).

2.3 DETECTION AND CLASSIFICATION OF *GIARDIA*

2.3.1 Detection Methods

The ability to accurately identify the sources of foodborne or waterborne human infections requires efficient detection methodologies. In clinical laboratories, microscopy is usually the first choice for the diagnosis of parasites. *G. intestinalis* can be detected by trained microscopists while performing routine ova and parasite examination of human clinical specimens, or stool samples of domestic animals. The cysts of *G. intestinalis* are oval shaped, and measure approximately 8–12 μm long and 7–10 μm wide.

The trophozoite has unique morphological characteristics that are easily visualized. It is shaped like a split pear, rounded on the anterior end and pointed posteriorly, convex on its dorsal side and the ventral region resembles a concave disk. Two nuclei are symmetrically located on each side of the midline of the trophozoite, giving the appearance of a face. Four pairs of flagella originate from the midline between the nuclei and are directed backward. The trophozoites of *G. intestinalis* are very fragile

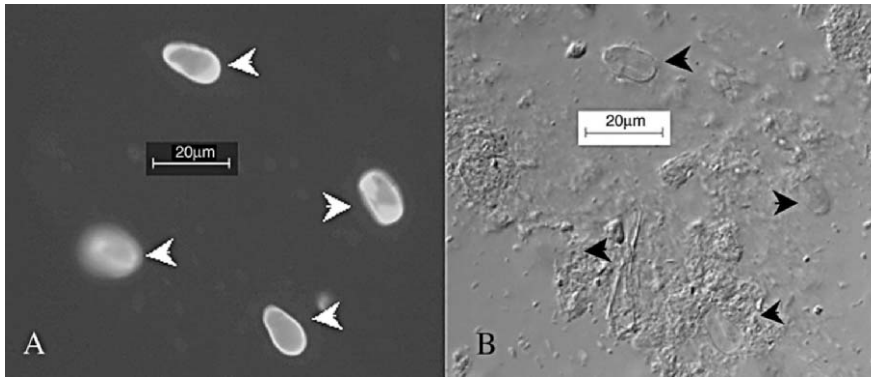

Figure 2.1. *Giardia intestinalis*, 400× magnification. A. Immunofluorescent microscopy. B. Nomarski microscopy.

and highly susceptible to changes in osmotic pressures, thus can be easily destroyed during sample processing. Whenever this stage is sought, microscopic examination of direct wet mount slides is the method of choice.

For the clinica diagnosis of *Giardia*, it is recommended to examine the stool specimens obtained from three consecutive days. Otherwise, diagnostic results cannot be assured. Despite these guidelines, it is estimated that about 30% of infections still remain undetected (Wolfe, 1990). An advantage of direct microscopy is its simplicity, low cost, and high specificity at the genus level. Since a positive microscopy result is usually correct, negative results, however, may require additional testing, especially if the gastrointestinal symptoms persist and no other etiologies are identified. The detection of *G. intestinalis* has been significantly improved with the development of immunofluorescent antibody microscopy (IFA) and enzyme immunoassays (EIA). The IFA method uses fluorescent-labeled antibodies that react with *Giardia* cysts, enhancing and simplifying the visualization of the parasite (Fig. 2.1). This method is of significant help for the untrained clinical microscopist, although its contribution has been more significant in the detection of parasites in environmental and food samples.

Clinical laboratories use either microscopy or EIA methods for detecting *G. intestinalis* in human specimens. Regardless of the diagnostic approach, it is imperative to test at least three stool samples per patient. When comparing these methods, neither is substantially better than the other. Light microscopy does not require any special reagents, is simple, and has a very high specificity. Meanwhile, EIA methods are better suited for processing large batches of samples, some of which can be quantitative and can be automated. In these settings, EIAs are more widely used, especially under the format of enzyme-linked immunosorbent assays (ELISA) that can detect soluble *Giardia* antigens in stools (Ungar *et al.*, 1984).

In contrast to the clinical specimens, the microscopic detection of *Giardia* cysts in source or finished water and in fresh produce is still a significant challenge. Currently, testing for the occurrence of *Giardia* in surface water to be used by drinking water treatment plants is mandatory under the 1996 amendment of the *Safe*

Drinking Water Act. To meet this mandate, in 1999 the US Environmental Protection Agency (EPA) implemented a validated procedure that involved immunomagnetic recovery of *Giardia* cysts followed by IFA microscopy. The test is known as US EPA Method 1623 for the detection of *Giardia* and *Cryptosporidium*. As a consequence, there has been an increase in the availability of validated tests for the detection of *Giardia* cysts and *Cryptosporidium* oocysts. While these tests were developed for water testing, several of these methods have been adapted for detecting both parasites in food matrices.

In summary, microscopy and ELISA/EIA are the methods most commonly used in clinical laboratories. Meanwhile, immunoassays and IFA are most frequently used for detecting *Giardia* in environmental and food samples.

2.3.2 Classification of *G. intestinalis*

Serology or typing based on immunological reactivity has been commonly used for typing the bacteria. This method, however, has demonstrated poor diagnostic differentiation for most parasites, including the *G. intestinalis.*

Zymodeme analysis, more accurately described as multilocus enzyme electrophoresis, identifies differences in enzymes that are the result of amino acid substitutions. Panels of 15–20 different enzymes are tested to identify differences in the charges of the native enzymes and can usually yield at least one with a difference. Nonetheless, this method is limited to selected research laboratories since it requires a large number of organisms.

In recent years, differences in nucleotide sequences have been more widely used to understand the diversity of an organism. One alternative is by identification of specific gene sequences that can be visualized in the form of restriction fragment length polymorphisms (RFLPs). This approach has been used for classification of bacteria and has proven very useful with some protozoa. One of the methods for detecting RFLPs is the digestion of DNA by an infrequently digesting restriction enzyme, followed by electrophoretic separation of the digested fragments in agarose gels, resulting in patterns that can be used for genotyping.

Pulsed field gel electrophoresis (PFGE) has been used for epidemiologic investigation of outbreaks of bacterial infections (Lipuma, 1998; Maslow *et al.*, 1993). However, PFGE requires relatively large amounts of DNA, and thus it has limited application in the typing of noncultured protozoa. Nonetheless, PFGE without enzyme digestion has been successfully used with *G. intestinalis* (Adam *et al.*, 1988) and *Cryptosporidium parvum* (Blunt *et al.*, 1997; Caccio *et al.*, 1998; Hays *et al.,* 1995; Mead *et al.,* 1988). Despite the vast amounts of DNA required, its results do not always correlate well with genotypes.

Recently, a polymerase chain reaction (PCR) followed by RFLP was used for the detection and subsequent genotype determination of *Giardia*. A significant advantage is that small amounts of *Giardia* DNA are required, while the PCR-amplified products can be analyzed using RFLP (Caccio *et al.*, 2002). Another DNA amplification method, such as random amplified polymorphic DNA, also requires little DNA and does not require prior knowledge of DNA sequence.

Most molecular methods based on PCR require a very little amount of DNA and can be quantitative in their comparisons. During the past few years, the cost of these

methods, including sequencing, have drastically decreased while the quality has improved, making this a highly attractive method of classification and even detection. As with all DNA-based methods, a potential limitation is finding adequate gene targets that are easily amplifiable by PCR. Additionally, these PCR products should have sequence polymorphisms that can be utilized to differentiate closely related organisms, but most importantly, those with human or animal infectious potential.

To date, the isolates of *G. intestinalis* have been classified into two major genotypes or assemblages, A and B (Adam, 2000). Assemblage A comprises groups 1 (genotype A-1) and 2 (genotype A-2) previously described by Nash. These two groups can be differentiated readily by molecular techniques (Baruch *et al.*, 1996; Lu *et al.*, 1998). Genotype B (Nash group 3) is very different from genotype A, having 19% nucleotide divergence for the TPI gene (Lu *et al.*, 1998) and 13% difference for the ADP-ribosylating factor gene (Murtagh *et al.*, 1992).

2.3.3 Genotyping of *G. intestinalis*

The use of molecular tools not only confirms the validity of a species, but can identify specific subpopulations, also described as genotypes or assemblages. Recent molecular studies have confirmed distinct genotypes within *G. intestinalis* (Homan *et al.*, 1992; Maryhofer *et al.*, 1995; Monis *et al.*, 1999; Nash and Mowatt, 1992; Sulaiman *et al.*, 2003; Thompson *et al.*, 2000) (Table 2.1), and two major groups of human-pathogenic *G. intestinalis* have been recognized worldwide (Homan *et al.*, 1992; Maryhofer *et al.*, 1995; Nash *et al.*, 1985; Nash and Mowatt, 1992). Furthermore, various phylogenetic studies based on the characterization of nucleotide sequences of glutamate dehydrogenase (GDH), elongation factor 1α (EF1α), SSU rRNA, and TPI genes have demonstrated the existence of five to seven defined lineages of *G. intestinalis* (van Keulen *et al.*, 1991; Mowatt *et al.*, 1994; Monis *et al.*, 1996, Monis *et al.*, 1999; Sulaiman *et al.*, 2003).

Among the genes analyzed thus far for *Giardia* isolates from various hosts, the highest degree of polymorphism was observed at the TPI locus in a study conducted on 4 human isolates, 2 mice isolates, and 1 isolate each from cats, dogs, pigs, rats, and blue herons (Monis *et al.*, 1999). Later, a new TPI-based nested-PCR protocol was developed to amplify the TPI fragment from various *Giardia* isolates using primers complementary to the conserved published TPI nucleotide sequences of various *Giardia* parasites downloaded from the GenBank (Sulaiman *et al.*, 2003). Using this new nested protocol, a larger sample including 37 human isolates, 15 dog isolates, 8 muskrat isolates, 7 isolates each from cattle and beavers, and 1 isolate each from a rat and a rabbit (Sulaiman *et al.*, 2003) revealed that phylogenetic differences at the TPI gene was largely in agreement with all the previous results based on other genes (Monis *et al.*, 1996, 1999; Mowatt *et al.*, 1994; van Keulen *et al.*, 1991). This TPI-based phylogenetic study revealed the following groupings of *G. intestinalis* parasites: (i) a group containing only human isolates (assemblage A); (ii) a major group containing human muskrat, beavers, and rabbit isolates (assemblage B); (iii) a group containing isolates from cattle and pigs (assemblage E or the Hoofed livestock genotype); (iv) a group containing isolates from dogs (assemblage C); (v) a cat genotype; and (vi) a genotype from rats (Monis *et al.*, 1999; Sulaiman *et al.*, 2003).

Table 2.2. Distribution of assemblage A and B in humans reported in some previous studies.

Location	*Number of positive samples examined*	*Assemblage of samples (%)*			*Reference*
		*A**	*B***	*Mixed*	
Australia	13	100	0	0	Hopkins *et al.*, 1997
Australia	11	36	64	0	Andrews *et al.*, 1998
Netherlands	24	50	50	0	Homan *et al.*, 1998
Germany	12	92	8	0	Karanis and Ey, 1998
China	3	0	67	33	Lu *et al.*, 1998
Australia	4	50	50	0	Monis *et al.*, 1999
China	8	50	50	0	Yong *et al.*, 2000
Korea	7	100	0	0	Yong *et al.*, 2000
The Netherlands	18	50	50	0	Homan and Mank, 2001
China, Cambodia, Australia, USA	9	56	44	0	Lu *et al.*, 2002
United Kingdom	33	27	64	9	Amar *et al.*, 2002
Italy	30	80	20	0	Caccio *et al.*, 2002
Mexico	22	100	0	0	Ponce-Macotela *et al.*, 2002
Mexico	26	100	0	0	Cedillo-Rivera *et al.*, 2003
New Zealand	5	100	0	0	Learmonth *et al*., 2003
India	10	0	100	0	Sulaiman *et al.*, 2003
Peru	25	24	76	0	Sulaiman *et al.*, 2003
United States	2	0	100	0	Sulaiman *et al.*, 2003

*(group I, II or Polish),
**(group III, IV or Belgian)

Remarkably, these findings have demonstrated that assemblage B contains host-adapted *Giardia* genotypes that infect humans and other mammalian species, thus suggesting the zoonotic potential of this genotype/assemblage. There is still some controversy regarding the transmission potential of *Giardia* from animals to humans (Thompson, 2000; Thompson *et al.*, 2000), since identical genotypes have been reported in humans and animals, supporting the zoonotic potential of this parasite.

Even though two major molecular groups of *G. intestinalis* have been recognized infecting humans worldwide (Table 2.2), there is no agreement in naming these genotypes, and the nomenclature varies by the location of the scientist involved. The following genotypes are reported in the literature: (i) Polish and Belgian genotypes in Europe (Homan *et al.*, 1992), (ii) Groups 1, 2, and 3 in North America (Nash *et al.*, 1985; Nash and Mowatt, 1992), and (iii) assemblages A and B in Australia (Maryhofer *et al.*, 1995). It may be several years before a consensus may be reached

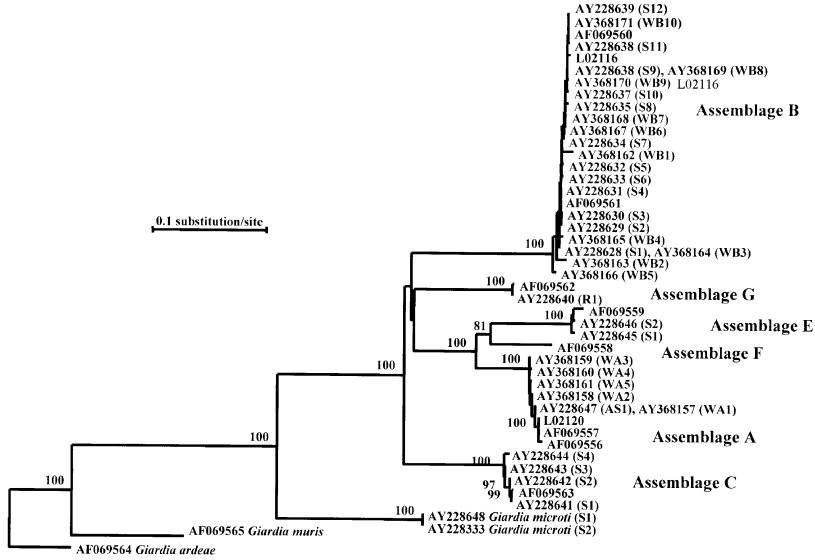

Figure 2.2. Phylogenetic relationships of *Giardia* parasites inferred by the neighbor-joining analysis of the TPI nucleotide sequences.

in naming *Giardia* genotypes; however, the term "assemblage" has been increasingly used by scientists worldwide, and will be used to describe the importance of genotyping.

Major advances in understanding the genetic diversity within *G. intestinalis* have been accomplished by the development of various PCR-based methods and protocols. Thus far, the TPI gene has been found to exhibit the highest degree of polymorphism in *G. intestinalis* at both inter- as well as intra-genotype levels, and the TPI-based genotyping has proven to be very useful in epidemiological investigations of human giardiasis (Monis *et al.*, 1999; Sulaiman *et al.*, 2003, 2004).

An interesting fact of *G. intestinalis* genotyping is that the phylogenetic distance separating assemblages A and B is greater than typically used to differentiate the two protozoan species (Fig. 2.2) (Monis *et al.*, 1996, 1998; Sulaiman *et al.*, 2003; Van Keulen *et al.*, 1998), reinforcing the notion that *G. intestinalis* may be a species complex. Several studies compared isolates of *Giardia* from different hosts as well as geographic regions and identified various genetically identical groups and subgroups within the *G. intestinalis* assemblages around the world (Monis *et al.*, 1999; Sulaiman *et al.*, 2003).

Both assemblages A and B of *G. intestinalis* have been reported worldwide, and these parasites have been found to be human-pathogenic in most continents (Table 2.2). The reported distribution of human-infectious genotypes, however, differed by study and geographical location. For example, a study in Mexico reported that all 22 isolates and derived clones from human specimens belonged to assemblage A subtype II, with a complete lack of assemblage B. The predominance of this subtype of assemblage A was attributed to biologic or geographic factors related to

the study area (Ponce-Macoteala *et al.*, 2002). Another study conducted in Mexico showed 26 human isolates belonging to assemblage A (Cedillo-Rivera *et al.*, 2003). Similarly, all seven human isolates in Korea characterized at 16S rDNA locus belonged to assemblage A of *G. intestinalis* (Yong *et al.*, 2000). However, in Peru and United Kingdom, assemblages A and B are found in human stools, although assemblage A was responsible for more human infections than assemblage B (Amar *et al.*, 2002; Sulaiman *et al.*, 2003).

A study in the United Kingdom of sporadic cases of humans giardiasis successfully used a TPI-based PCR-RFLP genotyping tool. Of the 33 TPI-PCR positive infected patients, 21 (64%) contained assemblage B, 9 (27%) had assemblage A, and 3 (9%) samples showed mixed infection of assemblages A and B (Amar *et al.*, 2002). Similar results were obtained with samples from a nursery outbreak, in which 88% (21 of 24) samples were shown to be *G. duodenlais* assemblage B parasites and rest of them to be assemblage A parasites (Amar *et al.*, 2002). Recently, the intra-genotypic variations of TPI in assemblage B were also considered as useful markers in subgenotyping outbreak isolates in a study conducted in the United States (Sulaiman *et al.*, 2003).

2.4 TRANSMISSION AND EPIDEMIOLOGY

Giardia species colonize the intestine of almost every group of vertebrates. *G. intestinalis* is the species that infects humans, domestic pets, farm animals, and wild mammals (Xiao *et al*., 1994; Olson *et al.*, 1995; Thompson *et al.*, 2000; Sulaiman *et al.*, 2003).

2.4.1 Human

In the United States, giardiasis is the most frequently detected parasitic disease. Results from a surveillance study from January 1992 through December 1997 revealed the occurrence of *Giardia* infections in all the major geographic areas of the United States, with an estimated incidence of 2.5 million cases per year (Furness *et al.*, 2000).

Recent molecular characterization studies indicate that there are many host-adapted *G. intestinalis* genotypes, and with the use of these molecular tools, it is now possible to assess the human infective potentials of the *Giardia* cysts found in the water system. Additionally, these methods would be beneficial for tracking sources of contaminations (Sulaiman *et al.*, 2003).

Although most human infections are thought to occur due to anthroponotic activities, drinking and recreational water, fresh foods, and certain mammals may also play significant roles in the transmission of *G. intestinalis* (Mead *et al*., 1999; Nichols, 2000). Additionally, misting during commercialization or industrial washing is another way in which fresh produce can be contaminated with *G. intestinalis*, and other parasites (Amahmid *et al.*, 1999).

The identification of *Giardia* as the causative agent in a foodborne outbreak is a daunting task. The long incubation time, usually between 2 and 3 weeks, the variable percentage of symptomatic infections, and the use of detection methods that do not

allow trace-back investigations, recall bias from the affected people and the lack of samples suspect food products at the time infections are almost invariably observed in a suspected foodborne outbreak of giardiasis. Nonetheless, there are outbreaks where fresh produce or uncooked foods were confirmed as the vehicles of infection (Schantz, 1991).

Giardia intestinalis has a ubiquitous presence in the environment and is detected worldwide. A review by CDC researchers (Mead *et al.*, 1999) estimated that every year 225,000 cases of foodborne giardiasis occur in the United States. Because giardiasis is frequently considered a water- or foodborne illness, the identification of *Giardia* at the genus and species levels is insufficient to establish its role in outbreaks.

The accurate identification and molecular characterization of foodborne parasites is critical for successful epidemiological and trace-back studies. Findings from these investigations can lead to the identification of sources or points of contamination, the magnitude of parasite dissemination, and to the elaboration of appropriate interventions to prevent future outbreaks. Additionally, it can also be used to evaluate the efficacy of current or new preventative measures to prevent future transmission.

2.4.2 Environmental

Waterborne outbreaks of giardiasis are a major public health problem in many industrialized nations, including the United Kingdom, Sweden, Canada, and the United States (Ljungstrom and Castor, 1992; Moore *et al.*, 1993). Human sewage has been considered a source of *Giardia* cysts contamination in water. In Canada and Italy, a high prevalence (73–100%) of *Giardia* cysts was reported in raw sewage samples (Caccio *et al.*, 2003; Heitman *et al.*, 2002; Wallis *et al.*, 1996). The public health importance and contamination sources of *Giardia* cysts found in water, however, are largely unclear, because very few studies have been carried out to genetically characterize the *Giardia* cysts in water. Nevertheless, *G. intestinalis* cysts of assemblage A have been identified in a few clams collected from the Rhode River, a Chesapeake Bay subestuary in Maryland (Graczyk *et al.*, 1999).

Molecular characterization of *Giardia* species in wastewater provides a valuable tool for community-wide surveillance of human giardiasis. Several attempts have been made to detect and differentiate *Giardia* species in environmental samples using PCR techniques for the detection and differentiation of *Giardia* parasites (Caccio *et al.*, 2003; van Keulen *et al.*, 2002; Wallis *et al.*, 1996). The distribution of *Giardia* species in environmental samples is likely dependent on human, agricultural, and wildlife activities. Two human-pathogenic genotypes of *G. intestinalis* (assemblage A in 10 and assemblage B in 4) were identified in 14 environmental samples (Wallis *et al.*, 1996) from Canada using a SSU rRNA-based PCR-RFLP protocol (van Keulen *et al.*, 2002). In Italy, 16 samples from four wastewater plants were analyzed by beta-giardin-based PCR-RFLP method. Assemblage A was found in eight of the samples, whereas both assemblages A and B were found in the remaining eight samples (Caccio *et al.*, 2003).

In a recent *Giardia* surveillance study, conducted on a much larger sample size of wastewater collected over a three-year period from an urban area of Milwaukee,

WI, both genotypes of human-pathogenic *G. intestinalis* (assemblages A and B) were found in the 131 samples (Sulaiman *et al.*, 2004). The majority (84.7%) of the wastewater samples (111 samples) belonged to *G. intestinalis* assemblage A, which had five distinct subtypes (WA1–WA5). However, one subtype (WA1) accounted for most of the assemblage A isolates (107 of 111), indicating humans in Milwaukee were infected with subtype WA1. This subtype was identical to a sequence previously reported in an axenically cultured strain from humans (AF069557, assemblage A, group II) in Australia (Monis *et al.*, 1999), and in six fecal samples from humans (AY228647) in Peru (Sulaiman *et al.*, 2003). The significance of a predominant subtype in Milwaukee is not clear. It is tempting to conclude that a common source of human infection was responsible for the wide occurrence of subtype WA1. However, the 20 wastewater samples that formed assemblage B had a high genetic diversity (with 10 distinct subtypes), indicating that it is unlikely the transmission of *Giardia* infection in Milwaukee is restricted to one source. It is possible that subtype WA1 of assemblage A is more infectious than other *Giardia* parasites (Sulaiman *et al.*, 2004). Recently, phenotypic differences between assemblages A and B have been observed; assemblage B was seen in patients with persistent diarrhea, whereas assemblage A was seen mostly in patients with intermittent diarrhea (Homan and Mank, 2001).

Phylogenetic analysis based on nucleotide sequencing has been successful not only in understanding the species structure of various microorganisms of public health importance, but also in identifying their transmission routes. Phylogenetic analysis based on all published *Giardia* TPI sequences from various previous studies, including *Giardia* isolates from humans, animals, and environmental samples, clearly demonstrated only assemblages A and B to be human-pathogenic parasites (Monis *et al.*, 1999; Sulaiman *et al.*, 2003, 2004) (Fig. 2.2). Thus, results of the phylogenetic analysis could be useful in understanding the public health importance of some *G. intestinalis* parasites. It is important to mention that the human *G. intestinalis* parasites belonged to only two distinct lineages (assemblages A and B), whereas four lineages contained the *G. intestinalis* from animals (assemblages C and E, and cat and rat genotypes). Assemblage B, however, also contains various animal isolates, such as all the beaver isolates and some isolates from muskrats, rabbit, and mice, strongly indicating that these animal *G. intestinalis* isolates have the potential to infect humans. Previously, it was suggested that *Giardia* parasites from beavers could be a source of infection among hikers and some waterborne outbreaks of giardiasis (Monzingo and Hibler, 1987;Thompson, 2000; Thompson *et al.*, 2000; Wallis *et al.*, 1984). Results of these recent studies have provided genetic evidence to substantiate these claims.

2.5 CONTROL AND TREATMENT

Since *Giardia* is primarily transmitted through the fecal-oral route, one of the major vehicles for transmission is contaminated drinking water. The water supply systems may become contaminated by the introduction of sewage or animal activity in their

watershed. *Giardia* cysts can be effectively removed and inactivated in water supplies by a combination of filtration and disinfection.

The filtration of drinking water supplies is accomplished by removing particulate matter from water by passage through porous media. A large number of filtration technologies are utilized for this purpose, including diatomaceous earth filtration, slow sand filtration, and coagulation filtration. There are several types of coagulation-filtration practices that include conventional filtration, direct filtration, and in-line filtration. It has been estimated that all of the above filtration methods can remove ≥99% of the *Giardia* cysts from raw water, provided they are operated and maintained properly (Logsdon, 1988). Since filtration can reduce, but not necessarily eliminate the levels of contaminants in water, disinfection is an additional process needed to ensure microbiologically safe water. This is particularly true when the source of drinking water is surface water.

It is indeed important to mention that approximately 14–15 million households in the United States depend on a personal household well for drinking water each year, and more than 90,000 new wells are drilled all over the United States each year (www.cdc.gov/ncidod/dpd/healthywater/privatewell.htm). During 1999–2000, contaminated private well water caused 26% of the drinking water-borne outbreaks, making people ill (www.cdc.gov/ncidod/dpd/healthywater/factsheets/wellwater.htm). Therefore, the contamination of a private well is not only the concern of the household served by the well, but also nearby households using other water supplies and the aquifer that the water is drawn from. Rules from the US EPA that protect public drinking water systems do not apply to privately owned wells. Most states of the United States have rules for private wells, but these rules may not completely protect the private wells. A guideline (as fact sheets) has also been posted in the above Web site of Division of Parasitic Disease, Centers for Disease Control and Prevention, Atlanta, providing information on contaminants that can be found in well water, and information on making well water safe to drink.

Giardia cysts from human or animal sources that contaminate surface water can survive well at temperatures below 20°C, and still can be infective to humans. Previously described filtration protocols can remove 99% or more of *Giardia* cysts, and various chemical disinfectants (namely chlorine, chlorine dioxide, chloramines, and ozone) can further reduce the cyst burden in drinking water. However, the success of these methods are dependent on several factors such as pH, concentration, and contact time.

In a recent review, 21 waterborne outbreaks on cruise ships were examined (Rooney *et al.*, 2004). During the period of January 1970 to June 2003, more than 6400 people were found affected. Importantly, many outbreaks were neither reported nor published. The study also revealed that the above outbreaks could have been prevented if water had been uplifted from reliable sources, and extra treatment (such as filtration and disinfection) as well as routine monitoring of residual disinfectants in distribution systems was accomplished on a regular basis (Rooney *et al.*, 2004).

Giardia continues to be an emerging pathogen in various epidemiologic settings. The PCR-based molecular tools are valuable in identifying routes and dynamics of

transmission, and species structure. Data from molecular epidemiologic studies using modern diagnostic tools will help prevent future outbreaks.

Control measures to prevent human giardiasis should be integrated with programs to prevent waterborne and foodborne pathogens. Personal control measures include paying special attention to personal hygiene, in order to prevent exposure to infectious feces. In the United States and other developed countries this is fairly simple to accomplish. Nonetheless, special attention must be given to day-care centers. Control and prevention of giardiasis in these settings require proper training and education of care providers as well as careful supervision of the disposal of fecal contamination. In places where an optimal sanitary infrastructure is not widely available, including areas in developing countries, environmental contamination with fecal material poses a significant challenge. In these circumstances, the best alternative is to educate people in personal hygiene habits and ways to protect their food and water used for their consumption. People facing those conditions, as well as campers and hikers, should boil, treat, or filter their drinking water prior to consumption.

Giardia cysts are very resistant to conventional water treatment, such as chlorination and ultraviolet irradiation. For large water systems, sand filtration or a similar method for physical removal, in addition to an effective disinfection treatment, can be a successful water treatment option.

For individual water supplies it is advisable to have the water tested prior to selecting a treatment option. Frequently used are combinations of filtration disinfection, and occasionally, reverse osmosis. To verify the efficacy of water treatment systems or point of use devices, visit the Web site of the National Sanitation Foundation (www.nsf.org).

In the event of infections, several prescription drugs are available to treat giardiasis. The cure rates of antigiardia compounds vary by the study, and range from 80 to 100%. Metronidazole (commercial name Flagyl) is recommended by the World Health Organization for chemotherapy. It is very effective and is the most commonly used drug in adult patients in the United States; however it has an unpleasant flavor and children do not tolerate it very well. As tolerance may be a problem in pregnant mothers and young children, furazolidone is recommended in expectant women (DuPont, 1989), although nausea or vomiting may still occur (Altamirano and Bondani, 1989). Furazolidone or nitoxozanide may be used for treating pediatric giardiasis. Additional drugs include tinidazole, a compound chemically related to metronidazole, and quinacrine. These two products are not available in the United States, although tinidazole is frequently used in other countries. Quinacrine is useful in the management of difficult cases and can be obtained through Panorama Pharmacy, Panorama City, CA (Nash, 2001).

Other products used to treat giardiasis include paramomycin and albendazole and metronidazole-related compounds such as ornidazole and secnidazole (Gardner and Hill, 2001). A more complete and up to date information regarding drugs against *Giardia* can always be found in the electronic versions of The Medical Letter (http://www.medletter.com) or the website of the Division of Parasitic Diseases, Centers for Disease Control and Prevention (www.cdc.gov/ncidod/dpd/parasites/default.htm).

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